MEDISAINS
Jurnal Ilmiah Ilmu - Ilmu Kesehatan

Original Article

Protective effects of *Gyrinops versteegii* leaf ethanol extract on Leydig cells and testosterone under excessive physical activity: an in vivo study

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ARTICLE INFORMATION

Received: November 03, 2025
Revised: January 22, 2026
Accepted: January 22, 2026

KEYWORDS

Antioxidants; Cell Count; Leydig Cells; Rats Wistar; Testosterone

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A B S T R A C T

Background: Excessive physical activity may lead to overtraining syndrome and increased oxidative stress, which can reduce Leydig cell count and testosterone levels. Gaharu (*Gyrinops versteegii*) leaf ethanol extract contains strong antioxidants that may counteract oxidative damage. However, the effects of excessive physical activity on Leydig cells and testosterone remain unexplored.

Purpose: This study aimed to evaluate the protective effects of Gyrinops versteegii leaf ethanol extract on male reproductive parameters under excessive physical activity conditions.

Methods: This experimental study employed a randomized, post-test control-group design. Twenty-five adult male Wistar rats were divided into five groups: normal control, excessive physical activity control, and three treatment groups receiving Gyrinops versteegii leaf ethanol extract at 100, 200, and 400 mg/kg BW. Leydig cell counts and serum testosterone levels were analyzed using ANOVA and correlation tests.

Results: The highest Leydig cell count and testosterone level were observed in the 200 mg/kgBW group (28.92 cells and 1.822 ng/mL, respectively). Significant differences in Leydig cell counts were observed among groups ($p<0.001$), and a positive correlation between Leydig cell number and testosterone levels was observed ($p=0.006$).

Conclusion: Gyrinops versteegii leaf ethanol extract demonstrated protective effects against reproductive impairment induced by excessive physical activity in vivo, with an optimal dose of 200 mg/kgBW.

INTRODUCTION

Excessive physical activity may induce overtraining syndrome and oxidative stress, characterized by excessive production of reactive oxygen species (ROS).[1,2] Elevated ROS can impair cellular integrity through inflammation, apoptosis, and mitochondrial dysfunction. Persistent oxidative stress has been associated with reduced Leydig cell numbers and decreased testosterone levels, which may contribute to male infertility.[3,4] Globally, infertility affects approximately 17.5% of adults, with male factors accounting for 20–30% of cases.[5] These findings are supported by evidence showing significantly increased ROS biomarkers in individuals undergoing high-intensity physical training.[6]

Leydig cell function and testosterone production are particularly vulnerable to oxidative stress induced by excessive physical activity. Although Leydig cells possess intrinsic antioxidant defense mechanisms, sustained oxidative stress may overwhelm these systems, leading to impaired steroidogenesis and spermatogenesis.[4,7] Previous studies have demonstrated that certain medicinal plants, when administered at appropriate concentrations, can attenuate oxidative stress and preserve Leydig cell integrity and testosterone production.[8]

https://doi.org/10.30595/medisains.v24i1.28611

Gaharu *(Gyrinops versteegii)* is a woody plant native to Southeast Asia whose leaves contain bioactive compounds, including flavonoids and tannins, with potent antioxidant activity.[9] The synergistic action of flavonoids and isoflavonoids has been reported to enhance Leydig cell function and testosterone synthesis, thereby supporting normal spermatogenesis and preventing testosterone-deficiency-related disorders.[10] In addition, Gyrinops versteegii leaves exhibit a high total phenolic content, indicating their potential role in protecting biological tissues from oxidative damage.[11,12] Flavonoid constituents have also been shown to promote Leydig cell repair and enhance steroidogenic activity.[13]

However, to date, no experimental study has specifically evaluated the in vivo protective effects of the Gyrinops versteegii leaf ethanol extract on Leydig cell integrity and testosterone levels under conditions of excessive physical activity. Therefore, this study aimed to evaluate the protective effects of Gyrinops versteegii leaf ethanol extract against reproductive impairment induced by excessive physical activity.

METHOD

Study Design

This study is an experimental study using a randomized, post-test control-group design.[14]

Study Site

The study was conducted at the Integrated Biomedical Laboratory Research Unit, Faculty of Medicine, Udayana University.

Animal Preparation

A total of 25 adult male Wistar rats *(Rattus norvegicus)* were used in this experimental study. The sample size was calculated using the Federer formula for animal experiments, requiring a minimum of five animals per group. The rats were selected by simple random sampling and randomly assigned to five experimental groups to evaluate the effects of different interventions.[14] The animals were 10–12 weeks old and weighed 170–200 g at baseline. Prior to the experiment, all rats underwent a 7-day acclimatization period and were confirmed to be healthy, active, and free of anatomical abnormalities. Animals that refused to eat during the study were excluded, and any animal that died during the experiment was considered a dropout.

Plant Materials and Extraction

Gaharu leaves were air-dried, powdered, and macerated in 5 L of 96% ethanol for 48 h at room temperature with occasional stirring, then filtered. The residue was re-macerated in 2.5 L of 96% ethanol for another 48 h and filtered. Combined filtrates were concentrated under reduced pressure using a rotary evaporator at 68°C to obtain a crude ethanol extract. The extract was prepared to yield doses of 100, 200, and 400 mg/kg BW and administered orally (0.5 mL, gavage) once daily for 28 days after induction of excessive physical activity in groups KP1, KP2, and KP3, respectively.

Experimental Procedure

Twenty-five adult male Wistar rats were randomly divided into five groups (n = 5 each): a normal control (N), a negative control (K) subjected to excessive physical activity (swimming at 30°C for 30 min, four times weekly) and given 0.5 mL distilled water for 28 days, and three treatment groups (KP1–KP3) exposed to the same activity and administered 0.5 mL gaharu (Gyrinops versteegii) ethanol leaf extract at doses of 100, 200, and 400 mg/kg BW, respectively, for 28 days. On day 29, blood samples were collected for serum testosterone measurement using ELISA. The animals were then euthanized, and the testes were harvested for histological analysis. Leydig cell counts were evaluated on Hematoxylin–Eosin-stained sections under light microscopy at 400× magnification.

Data Analysis

Numerical variables were first tested for normality; p-values>0.05 indicated normality, and the variables were subsequently analyzed using one-way ANOVA. The correlation between Leydig cell counts and serum testosterone levels was also evaluated. A p-value < 0.05 was considered statistically significant.

Ethical Consideration

This study was approved by the Research Ethics Committee of the Faculty of Medicine, Udayana University (Approval No. 2161/UN14.2.2.VII.14/LT/2023).

RESULTS

The highest Leydig cell count and testosterone level were observed in the 200 mg/kg BW Gaharu extract group, while the lowest were found in the distilled water group (Figures 1, Table 1). A significant positive correlation was identified between Leydig cell count and testosterone levels (p = 0.006). Histopathological analysis (Figure 3) showed abundant Leydig cells in the normal group, marked reduction in the distilled water group, and increased cell distribution in extract-treated groups, indicating a dose-related protective effect of the extract on testicular function.

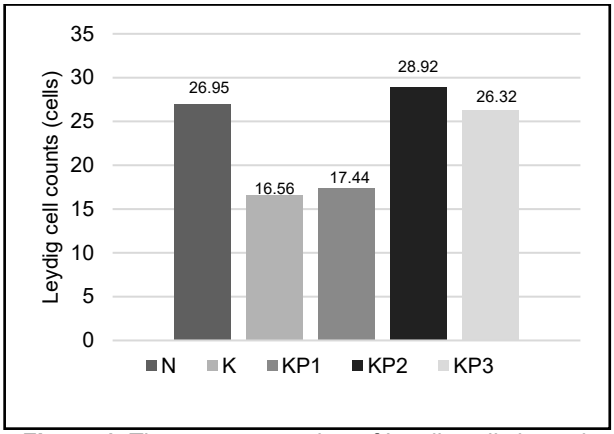

Figure 1. The average number of Leydig cells in each group

Table 1. Comparison of Leydig Cell Counts among Experimental Groups

Group	Treatment Description	Leydig Cell Count Mean ± SD	p-value
N	No treatment (Control)	26.95 ± 0.77	<0.001
K	Excessive physical activity and given distilled water	16.56 ± 3.54	
KP1	Excessive physical activity + 100 mg/kgBW Gaharu ethanol leaf extract	17.44 ± 4.68	
KP2	Excessive physical activity + 200 mg/kgBW Gaharu ethanol leaf extract	28.92 ± 2.75	
KP3	Excessive physical activity + 400 mg/kgBW Gaharu ethanol leaf extract	26.32 ± 2.06	

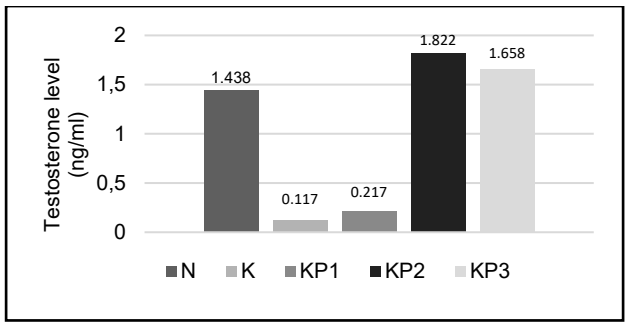

Figure 2. The average testosterone level in each group

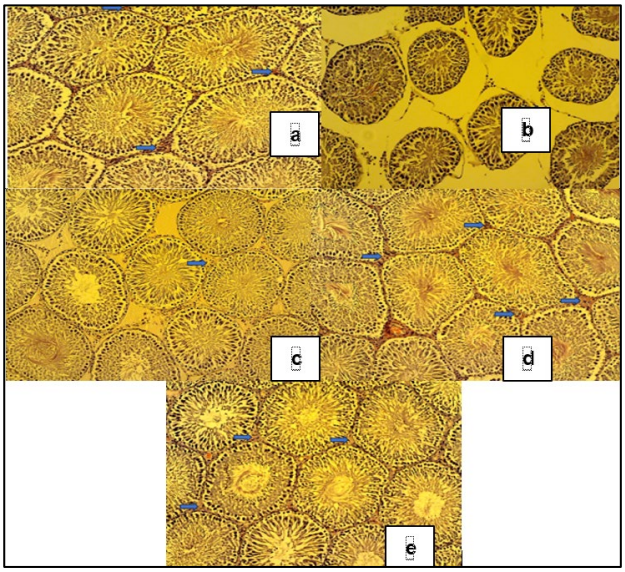

Figure 3. Histological images of testis sections show the number of Leydig cells in Wistar rats from each treatment group: (a) the untreated group, (b) the group given distilled water, (c) the group given 100 mg/kgBW of ethanol extract of Gaharu leaves, (d) the group given 200 mg/kgBW of extract, and (e) the group given 400 mg/kgBW of extract

DISCUSSION

This study demonstrated that ethanol extract of Gyrinops versteegii leaves inhibits the decline in Leydig cell count caused by excessive physical activity, with a statistically significant difference ($p < 0.001$) and an optimal dose of 200 mg/kgBW. A significant positive correlation between Leydig cell count and serum testosterone levels ($p = 0.006$) indicates a functional relationship between cellular preservation and hormonal production.

Previous studies have reported that Gyrinops versteegii leaves possess strong antioxidant properties, with high total phenolic content and low IC50 values, supporting their role in reducing oxidative stress.[11] Similar findings have shown that antioxidant-rich plant extracts can mitigate oxidative stress–induced impairment of Leydig cell function and testosterone synthesis in physically stressed animal models.[12]

These findings are consistent with previous studies reporting that higher Leydig cell numbers correlate with increased testosterone production mediated by luteinizing hormone (LH).[15,16] However, some studies have reported contrasting outcomes, where increased Leydig cell numbers were not accompanied by proportional increases in testosterone levels, suggesting that cell maturity and functional capacity also play important roles in steroidogenesis.[17]

Oxidative stress is known to impair Leydig cell function, reduce testosterone levels, and disrupt spermatogenesis, potentially leading to infertility.[18,19] These disruptions may impair Leydig cell activity and reduce testosterone production, contributing to reproductive dysfunction.[20] The bioactive compounds in *Gyrinops versteegii*, particularly flavonoids and phenolic constituents, may exert protective effects by reducing oxidative damage and supporting steroidogenic pathways.[21,22] Antioxidant-rich extracts, such as those from gaharu leaves, may therefore mitigate the adverse effects of excessive physical activity on reproductive function.[23]

The protective mechanism may involve inhibiting cyclooxygenase-2 (COX-2), preventing StAR gene suppression, and enhancing steroidogenesis.[13] Additionally, flavonoids may modulate testosterone levels by promoting Leydig cell proliferation, inhibiting 5α-reductase, and competitively binding to aromatase enzymes, thereby maintaining testosterone availability.[24–27] However, increasing the dose does not necessarily enhance therapeutic effects. High doses of antioxidants may disrupt redox signaling pathways and reduce physiological adaptations to exercise, potentially leading to reductive stress.[28,29]

To our knowledge, this is the first experimental study to demonstrate the protective effects of Gyrinops versteegii leaf extract on Leydig cell integrity and testosterone levels under conditions of excessive physical activity. This study has several limitations, including a small sample size and a relatively short intervention duration, which may limit the generalizability and long-term interpretation of the findings.

CONCLUSIONS AND RECOMMENDATION

Administration of Gyrinops versteegii ethanol leaf extract effectively prevents the decline in Leydig cell count and serum testosterone levels in adult Wistar rats subjected to excessive physical activity. The optimal protective effect was observed at a dose of 200 mg/kgBW. Future studies should investigate additional reproductive hormones, such as LH and FSH, to clarify the underlying mechanisms and evaluate whether the effects are mediated through the hypothalamic–pituitary–gonadal axis. Long-term studies are also recommended to assess the sustained effects and safety of the extract.

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